Fungal biodeterioration of color cinematographic films of the cultural heritage of Cuba

I. Vivar [a], S. Borrego [a], G. Ellis [b], D.A. Moreno [c,*], A.M. García [c]

[a] Archivo Nacional de la República de Cuba, Compostela 906 e/San Isidro y Desamparados, Habana Vieja 10100, Ciudad de La Habana, Cuba
[b] CSIC, Instituto de Ciencia y Tecnología de Polímeros, Juan de la Cierva 3, E-28006 Madrid, Spain
[c] Universidad Politécnica de Madrid, Departamento de Ingeniería y Ciencia de los Materiales, Escuela Técnica Superior de Ingenieros Industriales, José Gutiérrez Abascal 2, E-28006 Madrid, Spain

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A B S T R A C T

Until recently, cinematographic film was largely cellulose-triacetate-based. However, this material is highly susceptible to biodeterioration, thus placing historic film collections, an important part of the cultural heritage of many countries, at risk. In the present study, samples taken from several biodeteriorated color cinematographic films belonging to the collection of the Cuban Institute for Cinematographic Industry and Arts (ICAIC) were investigated. Infrared spectroscopy showed that all films were of the same composition, i.e., a gelatin emulsion coating one side of a cellulose-triacetate-based film support. The films were analyzed by environmental scanning electron microscopy and scanning electron microscopy to determine the degree of biodeterioration and the type of colonizing microorganisms. Significant fungal colonization was found on both sides of the films in all samples, with a higher concentration of fungi on the gelatin emulsion side. Epifluorescence microscopy of fluorochrome-dyed films demonstrated that some of the fungi were still active, indicating that the films under study, and probably others at the ICAIC, are at risk of further deterioration. Fungi were identified by molecular biology techniques. The fungi mainly responsible for the observed biodeterioration were those belonging to the genera *Aspergillus* and *Cladosporium*, although other genera, such as *Microascus* and *Penicillium*, were identified as well. In accordance with the findings described herein, the existing guidelines for the prevention and control of film biodeterioration are discussed.

1. Introduction

Cinema, popularly referred to as "the seventh art," is part of humanity's cultural heritage and, as such, should be conserved for future generations. However, celluloid, the material used to make cinematographic films, is vulnerable to both chemical and biological deterioration. An understanding of these processes is essential to the development of tools to aid us in the task of maintaining or recovering these culturally and historically significant icons.

A cinematographic film is composed of a flexible plastic support coated with a layer of photosensitive emulsion. Throughout cinematographic history, several flexible plastic supports have been used to manufacture professional motion-picture films, including cellulose nitrate (from 1889 to 1950), cellulose triacetate (from 1948 to 2000), and polyethylene terephthalate (from the 1990s to the present). The first nitrate-based supports were of very poor chemical stability, in addition to being a fire hazard. Triacetate supports met all the technical and safety requirements for professional motion-picture films and, since the early 1950s, have completely replaced cellulose nitrate. All cellulose-based supports are highly rigid; thus, in order to provide the required flexibility they are treated with various plasticizers, such as triphenylphosphate (Wypych, 2004).

Around the early 1970s, film archivists became aware of the serious decomposition of cellulose-triacetate-based cinematographic film, with the loss of many film images as well as entire films. This problem is immediately recognized when the film container is opened, by the release of acetic acid. Its characteristic odor accounts for what is known in the film world as the "vinegar syndrome." Acetic-acid-type degradation is largely responsible for the fading color in chromogenic emulsions, which are currently universally employed in cinematography, and for the deformation and stiffening of the material (del Amo, 2006). In addition, over time the plasticizer migrates, leaving obvious viscous liquid or crystalline deposits on the film's surface (Allen et al., 1988b).

* Corresponding author. Tel.: +34 91 3363164; fax: +34 91 3363007.
E-mail address: diego.moreno@upm.es (D.A. Moreno).

To address the problems of the "vinegar syndrome," cellulose triacetate (CTA) supports have recently been replaced by the polyester PET (polyethylene terephthalate), due to its exceptional physical properties as a safe cinematographic film support (Abrusci et al., 2004). Polyester-based films are less biosusceptible than those with CTA as the support. In fact, due to the inherent strength and flexibility of polyester, plasticization is no longer required. This is an important advantage, as these additives, in addition to their migration, have been identified as substrates for microbial colonization, including in the cellulose plastic materials used as film supports (Lourenço and Sampaio, 2009). However, CTA-based films constitute the bulk of historical film collections throughout the world (Abrusci et al., 2004).

The manufacture of CTA involves the acetylation of the hydroxyl groups on the poly(anhydroglucose) structural repeat unit of cellulose. The degree of substitution (DS) of cellulose acetate, i.e., the average number of acetyl groups per anhydroglucose unit, ranges from 0 in the case of cellulose to 3 in the case of CTA. Although the objective is to obtain a cinematographic film that is completely acetylated, in general, the DS of cinematographic CTA is around 2.7 (Abrusci et al., 2004). Various authors have reported that biodegradation is highly dependent on the DS, and in all cases the rate of biodegradation was shown to increase as the degree of the cellulose acetylation decreased (Buchanan et al., 1993; Samios et al., 1997).

The photosensitive emulsion that coats the cellulose support is made of gelatin, a polypeptide that forms a biodegradable substrate. In the case of black and white films, the emulsion consists of silver salts and other chemical products. The photosensitive layer of color films is somewhat more complicated, as it is composed of green, red and blue sensitive emulsion layers sometimes separated by clear gelatin interlayers. The gelatins used in film are distinguished by their production process and the nature of the raw materials. Conventionally, type A gelatins are produced from acid-pretreated pig skins, type B gelatins from alkaline-pretreated bovine bones, type C gelatins from alkaline-pretreated bovine and cattle hides, and type AB from acid-pretreated bovine bones (van den Bosch and Gielens, 2003). For cinematographic films mostly type-B gelatins are used (Abrusci et al., 2004).

Both the cellulose support and the gelatin emulsion in cinematographic films are susceptible to microbial attack, leading to biodeterioration. The biodeterioration of cinematographic films is the loss of images due to the biodegradation of photosensitive emulsion and/or the biodegradation of the plastic support. While the microbial contamination of black and white cinematographic films collected from the archives of the Czech Republic and from Spain has been studied (Opela, 1992; Abrusci et al., 2005), the biodeterioration of archived film is still relatively poorly understood. Moreover, chromogenic photographic materials appear to be more susceptible to fungal colonization than black and white materials (Lourenço and Sampaio, 2009), based on the observations of several professionals working with photographic collections, who found that color images are frequently more contaminated than black and white ones. The same general observation has been made for color cinematographic films.

In the present work, the biodeterioration of different color cinematographic films belonging to the Cuban Institute for Cinematographic Industry and Arts (ICAIC) was studied as part of the long-term effort to preserve these culturally valuable materials.

2. Materials and methods

2.1. Sampling

Biodeterioration was grossly observed on cinematographic films stored at the ICAIC and considered to be part of the Cuban cultural heritage. On first examination, it was clear that the films were contaminated by filamentous fungi, as colonies were observed between the loose coils at the beginning of the film spools as well as along the edges of the tightly pressed inner coils. From among the many color films, six were randomly chosen, representing different storage zones of the archive (Table 1). Films were filmed between 1890 and 1991. The archive did not have the right conditions for the preservation of the cinematographic films. Temperature and RH values were 31 °C and 65%, respectively. In September 2010, samples taken under aseptic conditions from the outermost ends of these films were transferred to the Biodeterioration Laboratory at the Technical University of Madrid (UPM), Spain. The samples were preliminarily observed using a stereomicroscope (SZX12, Olympus) to gain an initial impression of the state of the biodeterioration. Areas of interest chosen for further analysis were cut into small fragments using sterile scissors.

2.2. Analysis of the film composition

The composition of representative film fragments was studied by infrared (IR) spectroscopy using a Perkin Elmer *i*-Series IMAGE IR microscope equipped with a 16× Cassegrain objective, incorporating a germanium ATR crystal with a 100-μm diameter contact surface, and an HgCdTe detector, coupled to a Spectrum GX FTIR spectrometer. Spectra were recorded from various positions on both sides of the films at a spectral resolution of 4 cm^{-1} in the range of 4000–580 cm^{-1}, accumulating 20 scans at each sampling point.

2.3. Analysis of biofouling on films

2.3.1. Electron microscopy

Without previous preparation, the film samples were observed by environmental scanning electron microscopy (ESEM) using an INSPECT, QUANTA 200 scanning electron microscope operated at an accelerating voltage of 20–25 kV, in order to assess the degree of deterioration of the support and the type of colonizing microorganisms. Film samples were also observed with SEM. For the latter, the specimens were washed twice with Milli-Q sterile water, fixed with 2.5% glutaraldehyde in sodium cacodylate 0.01 M at 4 °C for 2.5 h, dehydrated with a series of alcohol–water rinses (20, 40, 60, and 80%), and then submerged in these solutions for 30 min at 4 °C. The fixed samples were maintained in a 100% alcohol solution at 4 °C. They were further processed in a critical-point procedure (CPD 030, BAL-TEC) followed by gold sputtering (SCD 005, BAL-TEC) and then observed under a scanning electron microscope (DSM 960, Zeiss) operated at an accelerating voltage of 15 kV.

2.3.2. Study of fungal viability with epifluorescence microscopy

The viability of the microorganisms colonizing the cinematographic films was determined based on epifluorescence microscopy observation of the samples. These were washed twice with Milli-Q

Table 1
Color cinematographic films under study from "The Cuban Institute for Cinematographic Industry and Arts".

Sample	Film title/code	Year
Film 1	*Papeles Secundarios*. Roll #4-3	1989
Film 3	*1425*.	1981
Film 4	*Leyenda*. Key – 21–1698 – II – A	1981
Film 5	*Nueve entradas de pelota*. Key – 21–198 – I – A	1985
Film 6	*Llamada al Sol*. Key – 21–1158 – II	1980
Film 7	*Mascaró, el cazador americano*. Key – 21–1866 – XII – A	1991

sterile water and then stained using a commercially available fluorescent stain, the LIVE/DEAD® BacLight™ Bacterial Viability kit (L7012, Molecular Probes), which distinguishes viable cells from dead ones on the basis of membrane integrity. The content of this product is identical to that of the LIVE/DEAD® FungaLight™ yeast viability kit (L34952, Molecular Probes) used for fungi, which gave very good results in the preliminary studies carried out on the aforementioned control film. The kit contains two fluorochromes: SYTO 9 and propidium iodide (PI). Each sample was covered with a 1:1 staining solution with a final concentration of SYTO 9 and PI of 3.41 and 6.01 μM, respectively. The samples were incubated for 15 min in the dark at room temperature, washed with Milli-Q sterile water, air-dried, and then visualized with an epifluorescence microscope (Axioskop 2, Zeiss, Germany). SYTO 9 and PI could be observed simultaneously by using a filter block (Zeiss 487709: excitation 450–490 nm, FT 510 nm, LP 515 nm). Microphotographs were obtained using a Canon EOS 40D digital camera attached to the microscope.

2.4. Isolation and identification of the fungi

2.4.1. Fungal isolation

To isolate the cultivable fungi responsible for degradation of the cinematographic film, a protocol was developed for their surface recovery. Thus, small fragments of each film were washed with 15 ml of Milli-Q sterile water, which, along with dilutions thereof, was used to inoculate three different culture media: PDA (CM0139, OXOID), Bacto™ malt extract agar (218630, Becton, Dickinson and Co.), and oatmeal agar (255210, Difco) in Petri dishes. The film fragment itself was then placed on a sterile dish and scraped on both sides using a sterile metal spatula, after which an additional 3 ml of Milli-Q sterile water was added. This rinse water and dilutions thereof were also used as inoculants for the three above-mentioned culture media. Finally, the film fragments were cut in half using sterile scissors and each fragment was placed directly, with one side face downwards, on Petri dishes containing PDA. All of the samples were incubated at 28 °C for 2–4 wk. The isolated fungal strains were stored in cryovials with 1.5 ml of 30% glycerol at −75 °C.

2.4.2. Fungal identification

Genomic DNA was extracted from the isolated fungi using the commercial DNEasy Plant mini kit (QIAGEN) following the manufacturer's instructions. The ribosomal region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene were PCR-amplified using the primers ITS1 and ITS4 (White et al., 1990). PCR was performed in a Veriti® Thermal Cycler (Applied Biosystems) with the following program: 5 min denaturation at 95 °C, followed by 35 cycles of a 1-min denaturation at 95 °C, 1 min annealing at 56 °C, a 90-s extension at 72 °C, and a final extension step of 10 min at 72 °C. All reactions were carried out in a 25-μl volume containing 12.5 μl of ReadyMix™ Taq PCR Mix with $MgCl_2$ (Sigma), 5 μl of DNA (ca. 10–30 μg ml^{-1}), 25 pmol of each primer, and 0.4 mM $MgCl_2$. The PCR products were analyzed by electrophoresis in 1% (wt/vol) agarose gels in 1× TBE buffer containing ethidium bromide (0.4 μg ml^{-1}) and then purified using the enzymatic treatment ExoSAP-IT® (USB) following the manufacturer's protocol.

Fungi were identified by sequencing of the respective rDNA fragment using the BigDye® Terminator v1.1 cycle sequencing kit (Applied Biosystems). Sequences were resolved in an ABI PRISM® 310 Genetic Analyzer following the manufacturer's instructions and then compared directly to all known sequences deposited in the databases of the National Center for Biotechnology Information (NCBI), using the basic local alignment search tool Megablast.

Sequences were aligned using the ClustalX software v. 1.81 (Thompson et al., 1997). Phylogenetic and molecular evolutionary analyses were conducted using MEGA (Molecular Evolutionary Genetics Analysis) v. 3.1 (Kumar et al., 2004).

3. Results and discussion

3.1. Composition of the cinematographic films

Infrared spectroscopy attenuated total reflectance spectra obtained from both sides of the film fragments showed that all films were of the same general architecture, consisting of a gelatin emulsion on one side (side A) of a cellulose-acetate-based film support (side B). Fig. 1a shows a characteristic spectrum obtained

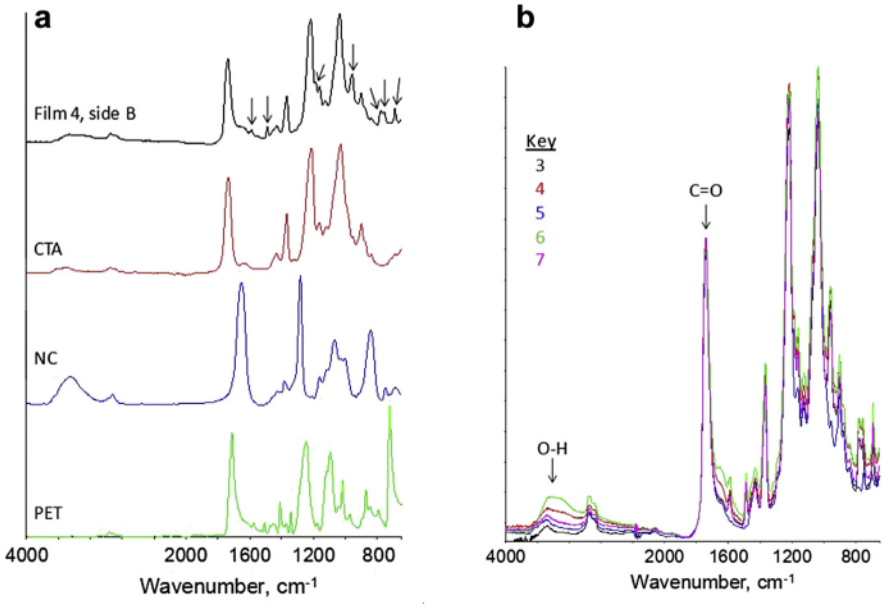

Fig. 1. (a) Infrared spectrum of side B of Film 4 compared with that of cellulose triacetate (CTA), nitrocellulose (NC), and polyethylene terephthalate (PET). (b) Normalized IR spectra from Films 3–7. The plots were shifted onto the vertical axis to facilitate comparisons.

from side B of one of the film fragments (Film 4) and, for comparison, the spectra of typical polymers employed in cinematographic film, including CTA, cellulose nitrate, and PET. The spectrum of Film 4 closely resembles that of CTA, with characteristic absorption bands of similar relative intensities at around 1737, 1367, 1216, and 1034 cm^{-1}, corresponding to C=O stretching, CH deformation, C–O–C stretching, and C–O stretching vibrations, respectively (Edge et al., 1989; Hanna et al., 1999). The low intensity of the broad OH stretching vibrations at around 3400 cm^{-1}, clearly observed in the spectra normalized to the intensity of the carbonyl stretch in Fig. 1b, confirmed the high degree of substitution of the acetate groups on the anhydroglucose unit. The relative intensity of the hydroxyl band compared to that of the carbonyl stretching vibration was previously shown to correlate with the DS, which in turn corresponds to the number of acetate groups. In that study, a calibration curve was established (Samios et al., 1997) that allowed us, using the same methodology, to obtain DS values >2.5 for all samples (Table 2), confirming that the support material of all the films studied was CTA.

A number of additional bands were also observed in the support film, notably those at around 1590, 1488, 1185, 957, 779, 756, and 690 cm^{-1}, marked on the spectrum in Fig. 1a. These were almost certainly associated with the presence of a plasticizer, which, as noted above, is necessarily incorporated into the film during its manufacture to improve its mechanical properties and to confer flame retardancy (Fordyce and Meyer, 1940; Ormsby, 2005). The bands observed in the film samples closely correspond to a plasticizer of the organophosphate family, but the presence of additional materials could not be ruled out. These bands exhibited similar relative intensities in all of the studied samples (Fig. 1b), with the exception of Film 5, in which case the aforementioned plasticizer was either absent, gradually lost, or substituted for by another type of plasticizer.

The degradation of CTA films is highly temperature- and humidity-dependent (Reilly, 1993). It occurs through an autocatalyzed hydrolytic de-esterification and a metal ion/acid-catalyzed degradation of the cellulose backbone (Allen et al., 1987). It is also influenced by the nature and composition of the plasticizer and the emulsion layer, both of which confer stability to the base film (Allen et al., 1988a). The biodegradation of plasticized cellulose acetate films, accompanied by the loss of the phthalate plasticizer, under conditions of >98% humidity, has previously been described (Jiang and Hinrichsen, 1997). However, the main consequence of CTA hydrolysis is a decrease in the bands associated with the loss of the acetate groups and a corresponding increase and shift in the broad OH stretching vibration at high frequency (Edge et al., 1989; Khatri et al., 2012). This was also the case in our IR spectra. Evidence for hydrolytic degradation of the film supports examined in this study is provided in the normalized spectra shown in Fig. 1b. In the cases of Films 4 and 6, the relative hydroxyl content was slightly higher than in the other films. Based on the assumption that the support material was CTA in all cases and that all films were maintained under the same environmental conditions, the changes in relative intensities corresponding to OH/C=O were interpreted as being a rough indication of the age of the films. In fact, as seen in Table 2, the spectral findings corresponded well with the respective dates recorded for the films.

Table 2
Estimated degree of substitution (DS) of acetate groups on the cellulose skeleton, calculated using the calibration of Samios et al. (1997).

Sample	Year	iOH × 10^3/aC=O	DS
Film 1	1989	–	–
Film 3	1981	1.07	2.78
Film 4	1981	1.81	2.60
Film 5	1985	0.98	2.81
Film 6	1980	2.15	2.51
Film 7	1991	1.04	2.79

With respect to the emulsion, although the spectra obtained from side A of the various film fragments were quite heterogeneous (Fig. 2), characteristic signals from gelatin were clearly observed in most of them. The most prominent of these signals was a strongly hydrogen bonded N–H stretching vibration with a maximum peak intensity at around 3280 cm^{-1}, as well as three amide signals (I–III) characteristic of gelatin: amide I, at 1634 cm^{-1}, largely due to the C=O stretching mode, with contributions from the N–H bending and C–N stretching vibrations of the amide group; amide II, at 1536 cm^{-1}, due to an N–H deformation mode; and amide IIII, at 1240 cm^{-1} (Sisson et al., 2009; Hashim et al., 2010; de Wael et al., 2011; Ibrahim et al., 2011).

Based on the FTIR data, the type of gelatin employed could not be determined. Comparing the spectra with those from type-A and type-B gelatin as reported in the literature also did not allow identification of the gelatin type used in the sampled film. A further complication was that the spectra of these two types are reportedly very similar (Hashim et al., 2010). Nonetheless, it was assumed that any biodegradation of the gelatin coating would also contribute to the highly heterogeneous nature of the spectra obtained. Corroboration of the conclusion that part of the surface contamination of the gelatin layer was due to the presence of fungi was obtained from several of the spectral signatures, particularly the broad band centered at around 1040 cm^{-1}. This band appeared in zones of the surface that were visibly contaminated at the microscale (marked * in Fig. 2), which, as reported by other authors, can be ascribed to

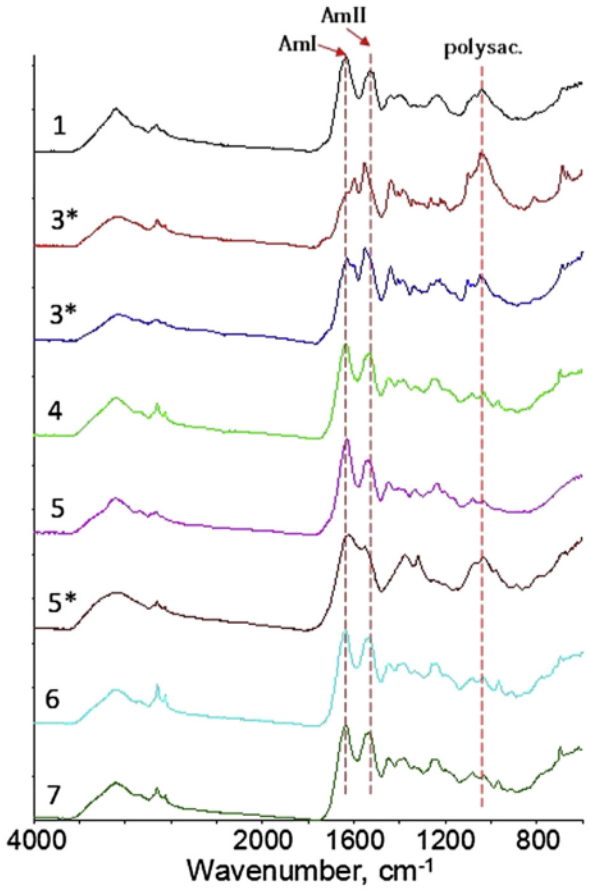

Fig. 2. Infrared spectra recorded from the emulsion side (side B) of Films 1, 3, 4, 5, 6, and 7 (note scale change at 2000 cm^{-1}).

polysaccharides in the cell walls of fungi (Bull, 1970; Michell and Scurfield, 1970; Fischer et al., 2006).

3.2. Biofouling study

Optical microscopy revealed signs of obvious biofouling and biodeterioration, albeit to varying extents, on all the films under study (Fig. 3). While Film 1 was completely covered in fungi, Films 4, 6, and 7 showed small brown stains. Based on this preliminary visual observation it was apparent that fungal development had occurred preferentially along the edge of the cinematographic film, in the vicinity of the film transport, rather than in the center of the film. This observation was not mentioned in the bibliography consulted, but clearly suggests that cinematographic film is more susceptible to fungal colonization in this area, possibly because contamination is easier and a greater amount of humidity is absorbed.

As demonstrated by SEM and ESEM, in all samples, a larger fungal colonization was present on the gelatin side than on the CTA side, although growth had also occurred on the latter (Fig. 4). Curiously, Lourenço and Sampaio (2009) similarly observed contamination on the cellulose nitrate side in a group of large negatives that had been stored stacked on top of one another. In our films, it seemed likely that fungal growth had begun on both the CTA side and the gelatin side, and was not a contamination of the former by the latter. The SEM and ESEM images confirmed the different degrees of colonization among the films analyzed, and that Film 1 was the most contaminated. On Film 6, not only was the support especially brittle (Fig. 5), but despite the fact that the samples had been washed twice with sterile water prior to SEM analysis, the fungi had remained strongly adhered to the film surface.

In addition to fungi, grains of pollen and mites were also present and had contributed to the biofouling of the cinematographic films.

Moreover, as seen on epifluorescence microscopy, although the proportion of living/dead fungi varied among the film samples, most of the fungi colonizing the cinematographic films were still active. This finding clearly showed that biodeterioration was an ongoing process of continuing harm to the supports under study (Fig. 6).

3.3. Identification of the isolated fungi

Thirty-seven different strains of fungi were detected on the color cinematographic film samples. Sequence analysis of the isolated fungi led to the identification of 14 different strains. The DNA sequences were deposited in GenBank under the accession numbers JQ409264–JQ409277. Table 3 describes the distribution of these fungi, along with an indication of their origin (rinse water, scraping, or fragment of cultured film) and their percentage of homology with the closest microorganisms stored at the NCBI. Among the fungi identified were different species of the genera *Aspergillus*, *Cladosporium*, *Microascus*, and *Penicillium*. *Aspergillus*, detected on three of the films studied, was the most abundant (72.97%), followed by *Cladosporium* (18.92%), *Microascus* (5.41%), and *Penicillium* (2.70%).

Aspergillus, *Penicillium*, and *Cladosporium* are commonly found in indoor environments such as houses, archives, libraries, and museums (Maggi et al., 2000; Górny et al., 2002; Borrego and Perdomo, 2012). For example, they were detected in the air in two film archives in the Czech Republic (Opela, 1992). In addition, *Penicillium*, *Aspergillus* sp., and certain species of *Alternaria* have often been found as contaminants or biodeterioration agents in many different habitats and materials, including those of historical and cultural heritage value. Among the known fungal strains, the majority exhibit cellulolytic, proteolytic, and/or amylolytic activities; they also produce acids, excrete different pigments, and contribute to the formation of biofilms, all of which accelerate substrate deterioration. Species belonging, e.g., to the genera *Penicillium*, *Fusarium*, *Aspergillus*, and *Trichoderma* are producers of cellulases (Itävaara et al., 1999), while the proteolytic activity of many fungal genera, including *Aspergillus* and *Penicillium*, was previously shown to result in the biodegradation of photographic emulsions (Abrusci et al., 2005; Bogomolova et al., 2007; Borrego et al., 2010). Of relevance to the ICAIC is the fact that the same *Aspergillus* and *Penicillium* genera isolated from the film samples were previously isolated from photographs stored at the nearby National Archive of the Republic of Cuba and, together with *Cladosporium*, were shown to be present in the air of that building (Guiamet et al., 2011).

Finally, it is interesting to remark that *Microascus* was observed for the first time on cinematographic films, although its cellulolytic activity has been known for many years (Verona et al., 1967). *Microascus* is the teleomorph of *Scopulariopsis*. Teleomorphs have been previously detected on documents (Borrego et al., 2010; Guiamet et al., 2011). This fungal genus is harmful to health because it can cause infections in toenails and eyes, skin lesions, respiratory disorders, and brain abscesses in immunosuppressed people (Baddley et al., 2000; Ustun et al., 2006; Issakainen et al., 2010).

3.4. Biodiversity of biodegrading fungi on cinematographic films

Abrusci et al. (2005) studied the microbial diversity on biodeteriorated cinematographic films in Spanish film archives, identifying a total of 17 strains of filamentous fungi and one type of yeast. *Alternaria*, *Aspergillus*, and *Penicillium* were the most frequently isolated genera but *Cladosporium*, *Mucor*, *Trichoderma*, and *Phoma* were also detected (Table 4). In the less humid environment of

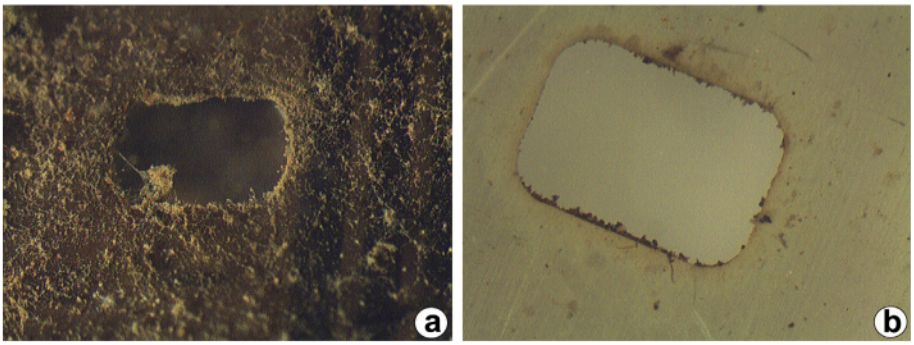

Fig. 3. Different degree of biofouling observed in the films under study. (a) Film 1 (×20) is completely colonized such that it is almost impossible to distinguish the edges of the perforation. (b) Film 4 (×20) is less biodeteriorated than Film 1.

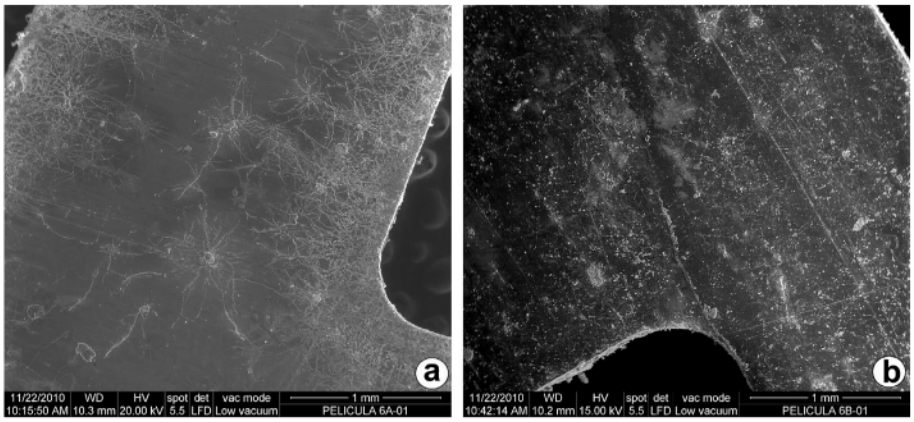

Fig. 4. ESEM micrographs showing fungal growth on Film 6. (a) Gelatin side, (b) cellulose triacetate side. Colonization is especially prominent on the edges of the film.

Madrid, *Aspergillus*, *Mucor*, and *Penicillium* predominated, while in the more humid environments of Barcelona and Gran Canaria the most abundant genera were *Alternaria*, *Clasdoporium*, *Trichoderma*, and *Phoma*. Thus, the biodiversity in the Abrusci study differed somewhat from that in our Cuban samples, most probably due to the significant climatic differences, i.e., a tropical island versus humid coastal areas and a dry, mountainous region.

Both *Aspergillus* and *Penicillium* are primary colonizers, since both can grow at low water-activity values ($a_w < 0.8$). Other fungal genera, including *Cladosporium*, require a_w values of at least 0.8–0.9. Therefore, in tropical climates, where the relative humidity (RH) is much higher, the widespread presence of *Cladosporium* is not surprising. By contrast, ours is the first reported observation of *Microascus* on cinematographic films, although its cellulolytic activity has been known for many years (Verona et al., 1967).

In the present study, all films showed fungal contaminants, albeit to varying degrees, with the most vigorous fungal growth occurring on Film 1 and the greatest biodiversity on Film 6 (Table 3). On Films 3 and 5, although fungal contamination could be observed by means of SEM and epifluorescence microscopy revealed living cells (Fig. 6), no fungal strain could be isolated using traditional culture methods. This suggests that the fungi on those two films were non-cultivable species. The use of PCR-based molecular tools on cinematographic films, rather than culture-dependent techniques, would no doubt have allowed the detection of many more microorganisms.

3.5. Biodegradation of gelatin by fungi

Gelatin is an animal protein that is easily attacked by microorganisms, and is even used to assay the proteolytic activity of different fungi and bacteria. As for other polypeptides, the biodegradation of gelatin involves the proteolytic hydrolysis of its peptide bonds. Fungi are highly capable of breaking down gelatin as they produce hydrolyzing gelatinases. The released amino acids are subsequently metabolized by the fungi as a source of carbon and nitrogen. Indeed, Abrusci et al. (2005) found that 17 strains of filamentous fungi isolated from biodeteriorated black and white cinematographic films stored in Spanish films archives were able to degrade gelatin in the test tube. Despite the presence of silver salts and other potentially toxic compounds in the emulsion of black and white images, the concentrations are apparently not high enough to inhibit fungal and microbial growth (Cserwinska and Kovalik, 1979). In color film, the dyes present in the emulsion are particularly vulnerable to the destructive effects of the microbial metabolites.

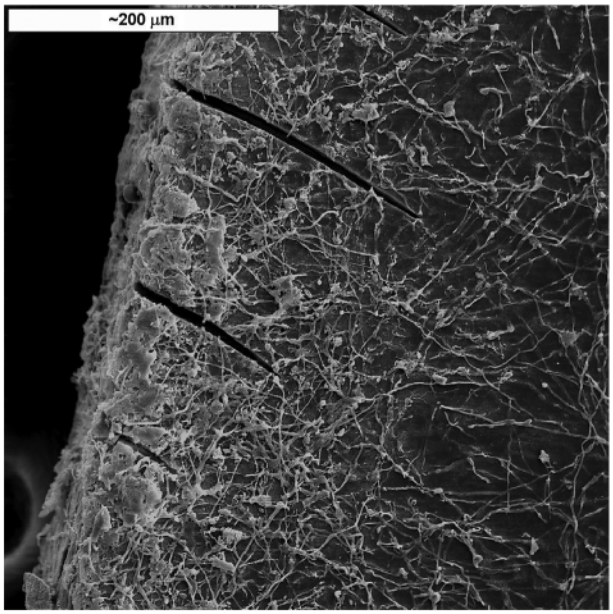

Fig. 5. SEM micrograph of a sample from Film 6, showing the edge of a perforation in this very brittle film.

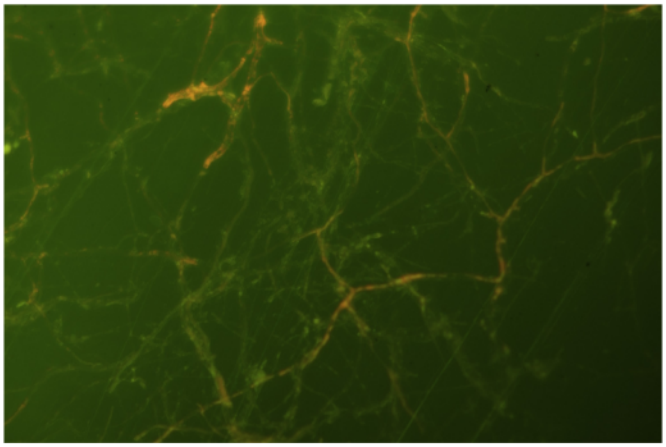

Fig. 6. Epifluorescence micrograph of Film 3 stained with SYTO 9 and PI. Fungal mycelia can be observed.

Table 3
Identification of fungi isolated from cinematographic films.

Strain code	Accession (NCBI)	Length (bp)	Number of isolates			Closest GenBank relative (NCBI)		
			Washing water	Scraping water	Film fragment	Strain or species	Accession	Identities (% homology)
7L1	JQ409277	506	Film 7 (1)		Film 7 (2)	*Aspergillus penicillioides*	GU017496	505/507 (99%)
1R7	JQ409273	538	Film 1 (1)	Film 1 (1)		*Aspergillus sydowii*	AY373869	531/534 (99%)
1L12	JQ409271	545	Film 1 (2)			*Aspergillus unguis*	FJ878626	544/548 (99%)
1R8	JQ409270	512	Film 1 (5)	Film 1 (2)	Film 6 (3)	*Aspergillus versicolor*	FJ878625	511/512 (99%)
1R10	JQ409272	457		Film 1 (1)		*Aspergillus versicolor*	FJ878625	439/458 (96%)
7.1.1	JQ409268	509			Film 7 (1)	*Aspergillus vitricola*	EF652046	437/448 (98%)
1L9	JQ409274	527	Film 1 (1)			*Aspergillus* sp.	JN709036	524/525 (99%)
1R4	JQ409275	546	Film 1 (3)	Film 1 (3)		*Aspergillus* sp.	HE608807	546/549 (99%)
1L3	JQ409276	406	Film 1 (1)			*Aspergillus* sp.	HQ832961	399/407 (98%)
6R5	JQ409265	524		Film 6 (5)		*Cladosporium phaenocomae*	JF499838	524/524 (100%)
6R1	JQ409264	497		Film 6 (1)		*Cladosporium* sp.	HQ829120	492/498 (99%)
6R6	JQ409266	478		Film 6 (1)		*Cladosporium* sp.	HQ829150	476/481 (99%)
4L1	JQ409267	451	Film 4 (1)	Film 4 (1)		*Microascus* sp.	HQ649896	430/455 (95%)
6R8	JQ409269	528		Film 6 (1)		*Penicillium chrysogenum*	JF440603	528/528 (100%)

3.6. Biodegradation of cellulose triacetate by fungi

Cellulose acetates are a class of natural polymers, with chemical modifications used to improve their processing features and mechanical properties for different applications. The natural cellulose backbone can have DS values ranging from zero to a theoretical maximum of 3, with the latter corresponding to full hydroxyl group substitution. While at DS values <2.5 cellulose acetate is susceptible to degradation (Gu, 2003), our results suggest that deterioration can also occur at higher DS values, ranging from 2.51 to 2.81. The degradation kinetics of polymers are known to be determined by the type and concentration of the structural substitution groups. Thus, cellulose acetate, with a relatively low DS (≈0.82), is more quickly degraded than polymers with higher substitution values (Buchanan et al., 1993). A lower DS implies a relatively high solubility, which favors microbial metabolism. Moreover, during the degradation of CA, both the molecular weight and the DS decrease, suggesting that de-acetylation and decomposition of the polymer backbone proceed simultaneously.

Filamentous fungi are efficient producers of extracellular cellulotytic enzymes (Wood, 1992), reflecting the fact that the first steps in depolymerization take place outside microbial cells, followed by the cellular uptake of the resulting oligomers for final mineralization. Given the availability of O_2, the final products of CTA biodeterioration are CO_2 and H_2O (Gu, 2003).

3.7. Prevention and control of biodeterioration in film archives

Cinematographic films stored in archives are exposed to the microorganisms present in the environment. Humidity and temperature conditions that are favorable for growth will allow these microorganisms to efficiently colonize the films, resulting in

Table 4
Fungi isolated from cinematographic films from different parts of the world.

Place	Spain (Abrusci et al., 2005)			Czech Republic (Opela, 1992)	Cuba (this study)
	M	GC	B	P	H
Weather conditions[a]					
Average annual temperature (°C)	15.25	21.25	16.70	9.50	25
RH (%)	58	62	72	75	76
Isolated fungi					
Alternaria alternata			X (2)		
Aspergillus nidulans var. nidulans	X				
Aspergillus penicilloides					X
Aspergillus sydowii					X
Aspergillus unguis					X
Aspergillus ustus		X	X		
Aspergillus versicolor		X		X	X (2)
Aspergillus vitricola					X
Aspergillus sp.					X (3)
Cladosporium cladosporioides			X	X	
Cladosporium phaenocomae					X
Cladosporium sp.					X (2)
Cryptococcus albidus			X		
Microascus sp.					X
Mucor racemosus	X				
Mucor sp.				X	
Penicillium chrysogenum	X	X (3)	X (3)		X
Penicillium frequentens				X	
Penicillium lanosum				X	
Phoma glomerata		X			
Trichoderma longibrachiatum		X			
Trichoderma viridiae				X	

M, Madrid; GC, Las Palmas de Gran Canaria; B, Barcelona; P, Prague; H, Havana.
[a] Calculated from data obtained from WeatherOnline (http://www.weatheronline.co.uk/).

their enzymatic degradation. Unfortunately, once this process has been initiated, there is, as yet, no satisfactory method to inhibit it. Nonetheless, knowledge and control of the aerobiology of film archives is fundamental in efforts to avoid the onset of biodeterioration of culturally valuable materials, including cinematographic films. Although the aerobiology of buildings housing cultural heritage, such as museums and libraries, has been frequently studied, there is very little information on film archives. As with any building, microorganisms enter from outdoors, by means of the building's ventilation systems, in addition to being transported inside by visitors and personnel. The surfaces and objects present within the building are also an important source of air contamination that, especially when the temperature and RH become optimal for microbial growth, will result in biodegradation, including of cinematographic films. The risk is greatest where the annual RH is well above 70%. As these climatic conditions prevail in tropical areas (Borrego et al., 2010), cultural heritage materials in countries such as Cuba are at particular risk.

Moreover, in their study of films in the National Film Archives (London), Allen et al. (1987) observed that the older the films, the higher the amount of moisture retained, which varied according to the degree of insolubility of the polymer. Thus, under archival conditions the slow absorption of moisture initiates hydrolytic degradation of the film, in turn enhancing the release of acetic acid, which accelerates the degradation process. This mild acidification favors fungal growth and together with the moisture results in significant biodeterioration.

Prevention is the only practical answer to the problem of microbial deterioration of cinematographic films. Such efforts must begin with a consideration of the storage conditions. The general recommendations of the International Federation of Film Archives (FIAF) and other authors are: nitrocellulose bases, 4 °C ± 1 °C and 50% RH; triacetate, black and white film, 21 °C and 20–30% RH; color film, 2 °C and 20–30% RH (Catalina and del Amo, 1999). For older CTA film, which already contains acetic acid, a lower temperature (−18 °C) would be more appropriate in order to freeze the acid (mp −17 °C) and decelerate the autocatalytic degradation process (Allen et al., 1987). However, these conditions are significantly more demanding than the standard conservation conditions in many films archives, i.e., 18 °C and 50% RH. Furthermore, they are very difficult to establish in warm, humid places such as at the ICAIC of Cuba, where, in addition, the necessary financial resources are lacking.

The Kodak Company, aware of the difficulties of decontaminating prints and films, established a series of guidelines to prevent fungal deterioration (Kodak, 2002). Thus, unexposed film should be stored in its original moisture-proof packaging and developed promptly after exposure, as improperly stored film, inside or outside the camera, is vulnerable to fungal degradation. However, while the fixing bath for black and white films should include a fungicide, these agents are not compatible with the dyes in color prints. Metal boxes should be used to store films, as wooden or cardboard boxes tend to absorb and hold moisture. A dehumidifier or air conditioner can be used to maintain an RH of 30–50%, appropriate for film storage; desiccants such as silica gel can cause the films to dry out. The films should occasionally be projected to increase dryness and keep them fungi-free. Clean cotton gloves should be worn to avoid contamination of the films.

4. Conclusions

The color cinematographic films stored at the Cuban Institute for Cinematographic Industry and Arts are contaminated by diverse species of fungi that result in severe biodeterioration. The isolated fungi belong to the genera *Aspergillus*, *Cladosporium*, *Microascus*, and *Penicillium*. The fungal biodiversity that affects cinematographic films based on a CTA support is limited to a few genera and varies according to the climatic conditions of the country where the films are archived. Therefore, it is crucial that cinematographic archives, such as museums and libraries, implement a microbiological quality-control strategy in order to prevent, or at least minimize, the risk of microbial colonization of cultural heritage material.

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